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Award Number: DAMD17-00-1-0360

TITLE: The Role of KSR-Associated Kinases in Breast Cancer Signaling

PRINCIPAL INVESTIGATOR: Steven J. Schreiner, Ph.D.
Robert E. Lewis, Ph.D.

CONTRACTING ORGANIZATION: University of Nebraska Medical Center
Omaha, Nebraska 68198-6810

REPORT DATE: February 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	February 2003	Annual Summary (1 Jan 02 - 1 Jan 03)

4. TITLE AND SUBTITLE
The Role of KSR-Associated Kinases in Breast Cancer Signaling

5. FUNDING NUMBERS
DAMD17-00-1-0360

6. AUTHOR(S)
Steven J. Schreiner, Ph.D.
Robert E. Lewis, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Nebraska Medical Center
Omaha, Nebraska 68198-6810

E-Mail: sschreiner@unmc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Kinase Suppressor of Ras (KSR) is a putative scaffold of the Raf/MEK/ERK kinase cascade. This kinase cascade is critical for the proliferation of malignant breast carcinomas. We have examined the effect of phosphorylation and protein-protein interaction on the subcellular distribution and biological activity of KSR. KSR is phosphorylated on at least 15 residues in intact cells. This phosphorylation is due to KSR-associated kinases and not due to autophosphorylation by the KSR kinase domain. Mutation of KSR phosphorylation sites reveals that phosphorylation of Ser392 and Thr274 potently inhibits the translocation of KSR from cytoplasm to nucleus. When R589M or C809Y mutations are introduced into KSR they prevent nuclear localization of KSR, inhibit the interaction of KSR with MEK, but enhance ERK activation and RasV12-induced anchorage-independent growth. Nuclear targeting of KSR by mutation of Ser392 and Thr274, or by the addition of the SV40 nuclear localization signal (NLS), does not alter the biological activity of intact KSR. However, addition of an NLS to KSR.C809Y accelerates cell proliferation in culture. These data suggest that the ability of KSR to affect cell proliferation and transformation is a function of its phosphorylation state, its interaction with MEK and its nucleocytoplasmic distribution.

14. SUBJECT TERMS
Kinase Suppressor of Ras (KSR), KSR-associated kinases, cdc-25-associated kinase1 (C-TAK1), protein scaffold,

15. NUMBER OF PAGES
5

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction

The cellular protein Ras integrates and transduces extracellular signals through protein-protein interactions of downstream effector molecules (1-4). One signaling pathway regulated by Ras is the Raf/MEK/ERK signaling cascade (5,6). Through genetic screening of defective Ras cellular signaling in Drosophila and Caenorhabditis elegans, KSR was identified as a positive regulator of Ras signaling events transversing the Raf/MEK/ERK pathway. Further studies have shown that KSR can have both a positive and negative affect on the ability of Ras to activate ERK MAP kinases (7-11). Evidence that KSR can interact with multiple members of the Raf/MEK/ERK signaling cascade has led to the suggestion that KSR may act as a molecular scaffold (7-10,12). Previous work from this lab has shown that KSR is phosphorylated on multiple amino acids in vivo, even though the kinase domain on KSR has been shown to be nonfunctional (13). This suggests that KSR interacts with other proteins, kinases, that are responsible for the phosphorylation and activation of KSR in cellular signaling. These sites of phosphorylation may form docking sites for other proteins, or induce conformational changes in KSR that regulate or coordinate the propagation of extracellular signals to ERK MAP kinases. Work performed under this grant has attempted to determine what other kinases or other effector proteins interact with KSR thereby affecting Ras signaling.

Body

Task 1 of the award was to identify proteins associated with KSR. Work under this award has resulted in the finding that the protein FHL3 interacts with KSR in two-hybrid screens performed with KSR fusion proteins and HeLa cytosolic extracts. Additional work found that the protein FHOS (Formin Homology Over-expressed in Spleen) also interacts with KSR. KSR was also shown to bind to the cytoskeletal protein vimentin, and to also bind and be phosphorylated by the protein kinase C-TAK1 (cdc-25C-associated kinase-1). Task 2 of the award was to determine the role of associated proteins in KSR signaling events. KSR is phosphorylated on at least 15 residues in intact cells. The protein kinase C-TAK-1 was shown to phosphorylate KSR in vivo on Ser392. The C-TAK1 binding mutant, KSR.V397A/V401A, and the point mutant KSR.S392A are not phosphorylated on Ser392, and result in an increase in nuclear localization of KSR, and cause cells expressing these mutant forms of KSR to grow at a faster rate and to a higher density than those expressing wild-type KSR. The interaction between FHL3 and KSR suggests that KSR, through interaction with CREB, may impact CREB-responsive gene transcription downstream of Ras signaling. Formin Homology (FH) proteins link cellular signaling pathways to the actin cytoskeleton and serum response factor-dependent transcription. This suggest that interactions between KSR and the cytoskeleton may be required for proper signal propagation through KSR. In a similar way, the finding that vimentin interacts with KSR further suggests a role for the cytoskeleton in Ras/KSR signaling. A putative site in KSR has been found that may be required for the KSR - vimentin complex to form. Taken together, these data suggest that the ability of KSR to affect cell proliferation and transformation may depend not only upon its phosphorylation state, but also by its interactions with other proteins including FHL3, FHOS, C-TAK1 and vimentin.

Key Research Accomplishments

- The protein FHL3 was shown to interact with KSR in two-hybrid screens performed with KSR fusion proteins and HeLa cytosolic extracts.
- The protein FHOS (Formin Homology Overexpressed in Spleen) forms a stable complex with KSR.
- Disruption of the C-TAK1 binding site caused a loss of phosphorylation on Ser 392 of KSR, and positively influenced cellular growth and KSR localization to the nucleus.
- KSR was shown to bind to the cytoskeletal protein vimentin, and a putative site has been found that may be required for the KSR - vimentin complex to form.

Reportable Outcomes

- An abstract and presentation of this work was presented at the Era of Hope Breast Cancer symposium in Orlando Florida in September of 2002.

- Paul Beum Ph.D., the award PI from Jan 2001 – July 2002, has taken a post-doctoral position at the University of Virginia at Charlottesville, West Virginia.
- A series of cell lines have been generated: One cell line expresses a KSR construct that cannot bind C-TAK1. Another expresses a KSR protein that contains a substitution of Alanine for serine at the site of C-TAK1 phosphorylation. A cell line expressing a mutant KSR that lacks the proposed C-terminal 14-3-3 binding site is under development, as well as a similar cell line expressing only the C-terminal portion of KSR containing this mutation. A line expressing a KSR construct that cannot bind FHOS has also been developed. All cell lines are or will be undergoing analysis for generating biologically significant changes to Ras signaling and / or transformation.

Conclusions

The statement of work for this award contained two specific tasks. Task 1 was to identify KSR-associated proteins. Work has shown that FHL3 and FHOS bind to KSR. These are unique findings and open new avenues of research into the involvement of KSR in alternative signaling pathways, and suggest new ways in which cells may regulate Ras mediated mitogenic signaling. We have also shown that vimentin binds to KSR, adding a new dimension to the KSR story. KSR interaction with the cytoskeleton (through vimentin and FHOS) may be required for effective Ras signaling, and novel ways to attenuate activated Ras oncogenic signaling may exist through blocking these interactions. Finally, it has been determined that C-TAK1 binds to and phosphorylates KSR. Task 2 was to determine the role of KSR-associated proteins. Although the cellular roles of FHL3, FHOS, and vimentin are partially known, their involvement in Ras signaling through KSR is not. Further work with mutant KSR constructs will help to determine the requirement of cytoskeletal interactions by KSR in cellular transformation by Ras. The exact role of C-TAK1 in Ras signaling is still under investigation. Mutations within KSR have been made that inhibit either C-TAK1 binding or the phosphorylation of KSR by C-TAK1. Specific biological changes in cells expressing this construct have been documented, including an increased growth rate and growth to higher densities than cells expressing wild-type KSR. If it is found that C-TAK1 is required for proper Ras oncogenic signaling, it provides an additional target for attenuating Ras cellular transformation.

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